Final Report (OTKA K112336; László Virág, University of Debrecen)

The main aim of our grant was to investigate how PARylation regulates macrophage (MΦ) cell fates, namely differentiation, polarization into specific MΦ subsets and MΦ death. Major achievements of our work are summarized below.

1) Role of macrophage (MΦ) polarization in oxidative stress sensitivity of MΦs

We found that severe oxidative stress causes regulated necrotic cell death in macrophages, with the nuclear enzyme PARP1 serving as the main perpetrator of necrosis. Unlike in canonical PARP1-mediated cell death known as parthanatos, cell death in this model proceeds in the absence of AIF translocation. We observed that the bacterial cell wall product LPS (lipopolysaccharide) that induced an inflammatory (M1-like) phenotype, protects macrophages (MΦs) against H_2O_2-induced necrosis (Fig. 1) via downregulation of PARP-1 and upregulation of SOD2.

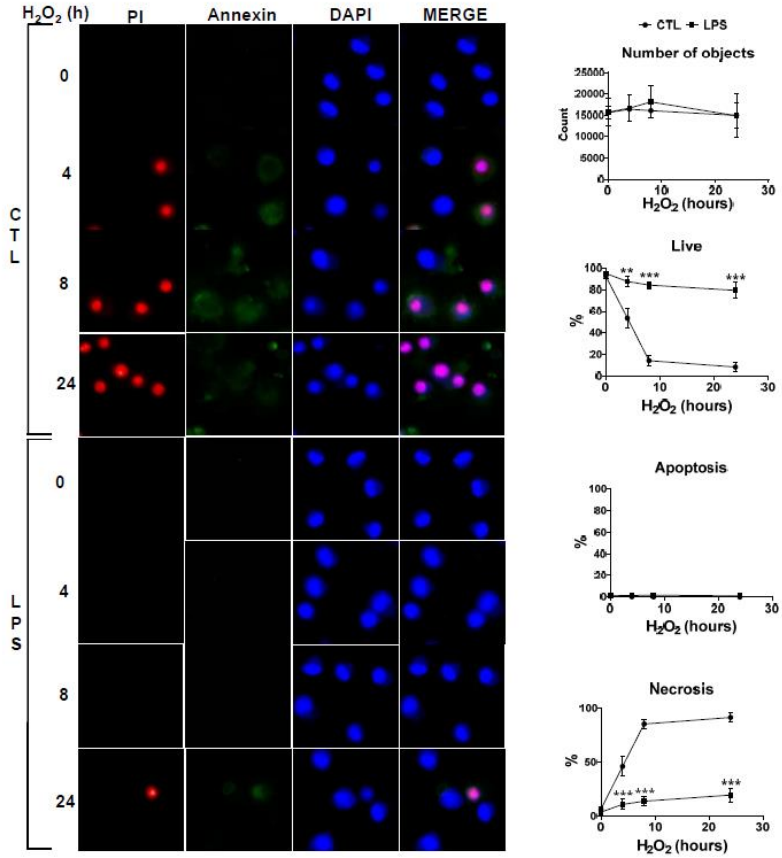

Figure 1. LPS protects macrophages from oxidative stress-induced necrotic cell death. Necrotic cell death was visualized after propidium iodide (PI) staining. Nuclei were stained with DAPI. (from Regdon et al FRBM 2019).

Downregulation of PARP1 expression in M1 like macrophages was also reflected in reduced PARylation capacity and preserved NAD pools (Fig. 2)

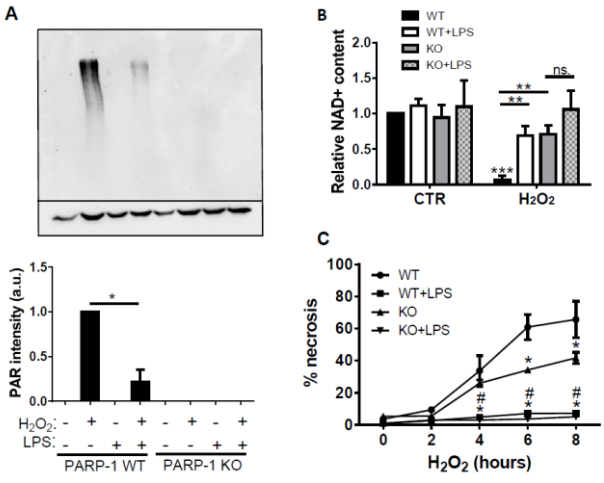

Figure 2. LPS-induced oxidative stress resistance is characterized by reduced PARylation capacity and prevention of NAD depletion. (from Regdon et al FRBM 2019).

To get further insight of the molecular mechanism of cell death in this model we used confocal microscopy to detect nuclear translocation of apoptosis-inducing factor (AIF). We did not detect nuclear AIF, however, both LPS and PJ34 protected against nuclear condensation suggesting that the nature of cell death is an AIF independent (i.e. non-canonical) parthanatos. Furthermore, metabolic changes are well known factors in the regulation of

redox homeostasis in cancer cells. To examine the metabolic phenotype of M1 MΦs we used Seahorse assays and ATP determination. LPS significantly increased the extracellular acidification and supressed mitochondrial respiratory capacity while PJ34 increased the maximal and reserved capacity of mitochondria. The ATP assay confirmed that both LPS and PJ34 treated MΦs have higher ATP content upon H_2O_2 exposure (Fig. 3).

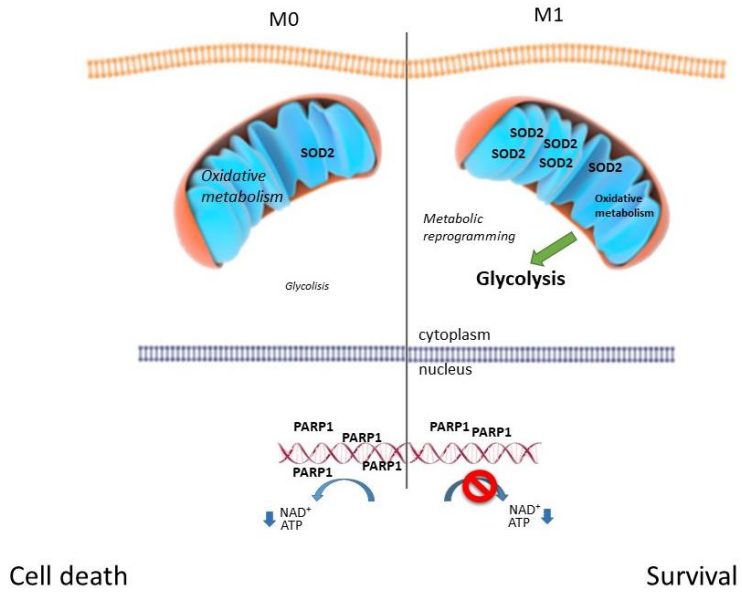

Figure 3. Activation of macrophages by LPS confers protection against hydrogen peroxide-induced cytotoxicity by downregulation of cell death mediators (e.g. PARP1, AIF), upregulation of antioxidant enzymes (SOD2, GPX1) and a shift to aerobic glycolysis. (from Regdon et al FRBM 2019).

As an extension of this project we characterized the mechanism potentially underlying the development of the protective M1 MΦ phenotype: in collaboration with Agnieszka Robaszkiewicz (Univ. Lodz, Poland) we described the protective role of SOD2 against H_2O_2 -induced cell death in human THP-1 cells. According to our findings PARP-1 functions as a transcriptional regulator inhibiting SOD2 expression. Upon LPS treatment PARP-1 is released from the SOD2 promoter increasing its transcription. (This additional paper also appeared in FRBM, 2019.)

The same collaboration also proved useful and productive in defining the role of PARP1 and Rbl2 interaction in monocyte gene expression. We found that Rbl2 regulates a sizable part of the proteome and a subgroup of this genes involves PARP1. Moreover, we also investigated the role of histone acetylation in this regulatory circuit. (This paper was published in BBA Gene Regulatory Mechanisms).

2) Role of PARylation in M2 polarization of macrophages

Our preliminary data suggested that PARylation regulates a subset of M2 MΦs genes. With extensive characterization of marker gene expression in IL-4 and IL-13 polarized M2 macrophages we established the role of PARylation in the M2 polarization of murine MΦs. Different PARP inhibitors were used, namely: olaparib, PJ34 and the PARP-14 selective MCD-113 to inhibit different members of the PARP family. These data suggested that the regulation is not via PARP1 but likely via PARP14. Moreover, we also set up a model to investigate how tumor cells reprogram MΦs. Conditioned medium of the mammary carcinoma cell line 4T1 (4T1 CM) was sufficient to polarize MΦs into an M2 phenotype. Different PARP inhibitors were used as described above. Olaparib did not affect the expression of M2 genes while high concentration (20 μM) of PJ34 and the PARP14 specific MCD-113 inhibited some tumor-associated macrophage-associated genes (arginase-1, IL4Ra, CCR2). PARP-1 knockout macrophages displayed normal M2 polarization so that all these findings point towards the possible role of PARP14 in this tumor-induced M2 reprogramming model (Fig. 4).

In order to verify the role of PARP14 in this model, we obtained PARP14 K.O. mice from collaborators and established a colony in our animal facility.

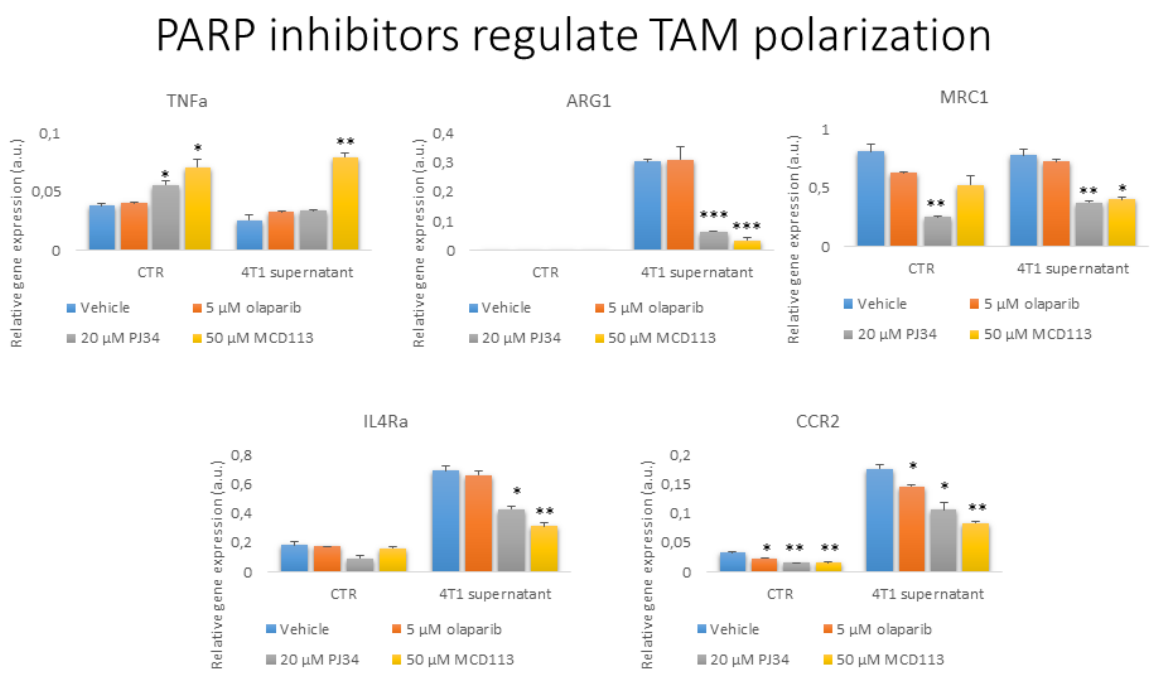

Figure 4. Effect of PARP inhibitors on 4T1 cancer cell-induced macrophage polarization markers

Once we had the PARP14 knockout colony, we set up genotyping of the mice and started doing experiments with macrophages obtained from them. M2 differentiation protocol was the same as described above for wild type MΦs. However, to our disappointment these cells did not recapitulate findings reported in the literature and these PARP14 deficient macrophages responded to IL4 and IL13 very similarly to wild type ones. Moreover, 4T1 conditioned media also induced an M2-like reprogramming similar to the wild type cells. These unexpected findings that are contrary to the literature, put these otherwise very promising project line on hold until we manage to resolve the roots of controversy. Nonetheless, the interaction between macrophages and different types of cancer cells was an attractive model to follow up, we set up several MF-cancer cell interaction models and plan to continue this new branch of research as part of a new OTKA project.

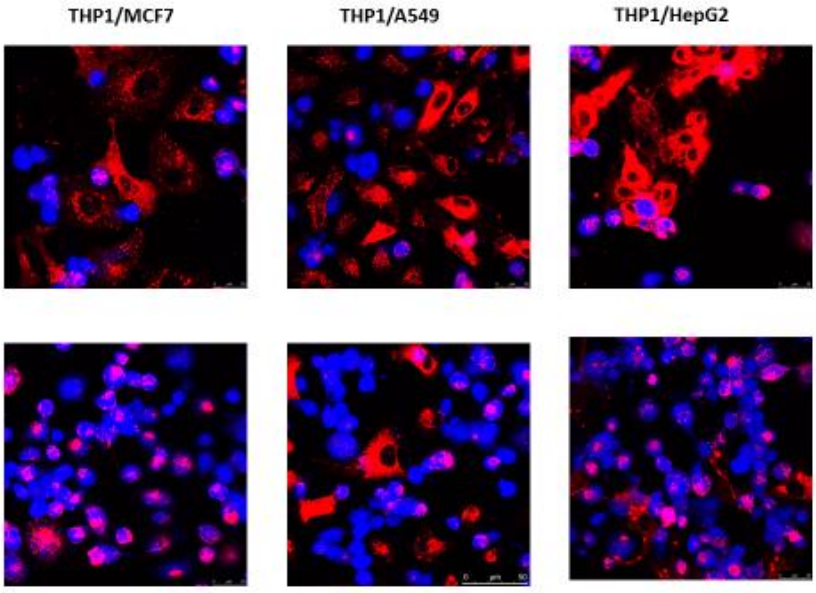

Figure 5. Trogocytosis in macrophage tumor cell co-cultures.

In this part of experiments we have incubated THP1 human leukemic monocyte-derived cells with different types of human carcinoma cell lines (MCF7: breast cancer; A549: human lung carcinoma and HepG2: hepatocellular carcinoma). Trogocytosis could be observed in the model and ongoing experiments investigate the mechanism of this complex interaction. (Fig.5)

3) Identifying M1 macrophage inhibitory natural compounds

We also performed experiments to investigate the effects of natural herbal anti-inflammatory substances on macrophage function. Acmella oleracea is a subtropical flower, which has long been used to treat inflammatory processes in the oral cavity. Several compounds had been identified in the extract of the plant, yet the bioactive substances and their precise effect on the native immune system remained unveiled. We could show that the organic extracts of the plant inhibited the NO production of INFγ and LPS-treated RAW264.7 macrophages in a concentration dependent manner without affecting cell viability. Next, we showed that suppression of the induction of iNOS was concomittant with the loss of NO production. Using chemically pure active ingrediants of the extract, we showed vanillic acid, trans-ferulic acid and scopoletin mildly suppressed NO production. Spilanthol, on the other hand, inhibited NO production markedly, improved cell viability at the same time, and had a suppressive effect on iNOS mRNA and protein levels (Fig. 6). We demonstrated by a transcription factor binding assay that the level of NFκB competent to bind its response element significantly decreases in the nuclei of spilanthol treated cells, which is likely to explain the mitigation of iNOS expression. Spilanthol prevented acute dermatitis in mice, decreasing swelling and inflammatory infiltration indicated by lowered MPO activity in the tissue. In the case of cerulean-induced pancreatitis i.p. spilanthol significantly reduced MPO activity measured from the pancreas (Fig.7). These experiments identify spilanthol as the major substance in Acmella suppressing RNS production by inflammatory macrophages, and provide clues to its molecular mechanism. We have summed up these results in a manuscript that is soon to be submitted for publication.

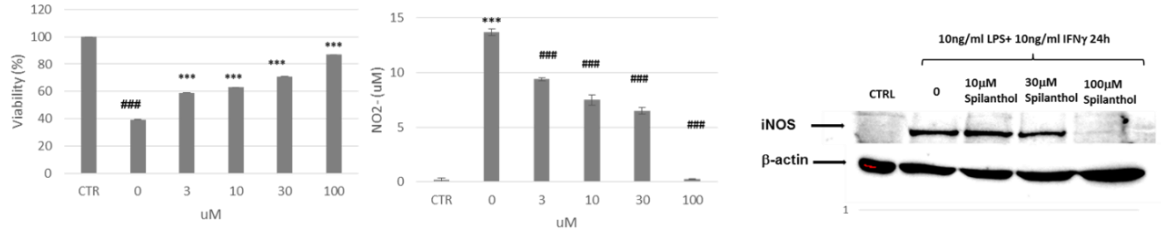

Figure 6. Effect of spilanthol on viability, NO production and iNOS expression in IFNγ/LPS treated MΦs. (From Bakondi et al. IJMS 2019)

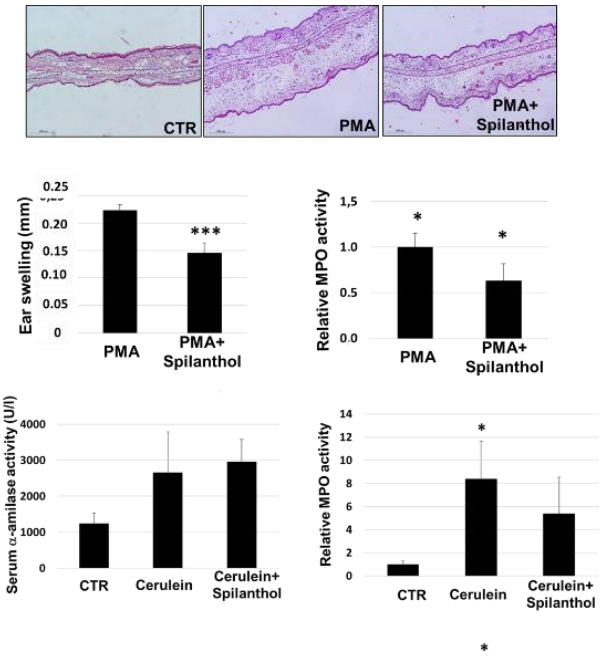

Figure 7. Inflammation suppressing effect of spilanthol in a PMA elicited acute ear dermatitis and a cerulein induced pancreatitis mouse model. (From Bakondi et al. IJMS 2019)

Various aspects of our work have been summarized in several review papers (see publication list appended.)